UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF FLORIDA

CASE NO. 00-6312-CR-ROETTGER

UNITED STATES OF AMERICA,)
)
vs.)
)
JERMAIN C. WILLIAMS, and)
LOWEN ESPINUEVA.)
)
Defendants.)

GOVERNMENT'S NINTH SUPPLEMENTAL RESPONSE TO STANDING DISCOVERY ORDER

The United States of America, in response to the Standing Discovery Order issued in this case file this ninth supplemental response which is alphabetized and numbered to correspond to that original order and states as follows:

A.6. See attached Curriculum Vitae of Jeffrey L. Leggitt and report on Fluorescein Detection and STR Typing; Curriculum Vitae of Brendan F. Shea and Curriculum Vitae of Jeffrey D. Grey.

Respectfully submitted,

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By:

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CERTIFICATE OF SERVICE

I HEREBY CERTIFY that a true and correct copy of the Government's Response to the Standing Discovery Order was mailed this 21 day of March, 2001 to: Darryl Wilcox, AFPD, 111 NE 3rd Ave, Ft. Lauderdale, FL. And Barry Wax, Esq. 201 S. Biscayne Blvd., Miami, FL. 33131.

THOMAS P. LANIGAN
ASSISTANT UNITED STATES ATTORNEY

cc: S/A Eric Miller, FBI

Curriculum Vitae

FBI Laboratory
Investigative Support Section
Structural Design Unit

935 Pennsylvania Avenue, N.W.
Washington, D.C.

Personnel Information:

Name-
Jeffery D. Gray

Title-
Visual Information Specialist

Responsibilities
Conducts crime scene surveys as preparation for the development of three dimensional scale trial and investigative models. Plans, designs and develops scale models, public liaison and training exhibits, special equipment concealment devices to aid investigative operations.

Tenure-
Have been employed by the FBI, in the same section, from August 26, 1979 to the present.

Education-
Bachelor of Arts; George Mason University, 1979. Major in Modern European History; Minor in French and Spanish.
Master of Arts; George Mason University, 1985. Major in Modern European History.

Specialized Training-
Autocad Level 1, Version 11; Fall 1990
Autocad Level 1, Version 13; Fall 1998
Autocad Level 1, Autocad 2000; Summer 2000
Autocad Level 1, Beyond the Basics; Summer 2000
Autocad 2000, 3D Drawing and Modeling; Summer 2000
College Level Introductory Course in Surveying; Catonsville Community College, Spring 1995
SOKKIA Set V Laser Transit Training; Spring 1996
SOKKIA Set V Laser Transit Training; Spring 1999
SOKKIA Set V Laser Transit Training; Summer 2000
Crime Scene Training, Quantico, Virginia; Fall 1988
Advanced Crime Scene Training, Quantico, Virginia; Spring 1997
Wordperfect 6.1
Gerber Training Level 1; Fall 1985
Gerber Training, Level 1; Spring 1998
Gerber Training, Level 1; Spring 1998
Gerber Edge Training; Summer 1999
Moot Court Level 1; Fall 1987
Moot Court Level 2; Spring 1988
Mastering Adobe Photoshop; Spring 2001

Work Experience-
Experience includes work on approximately 15 models, most of which have been for court use; have also been responsible for the design and construction of major exhibits for the FBI and other agencies and organizations.

CURRICULUM VITAE

NAME:

Brendan F. Shea

PROFESSIONAL ADDRESS:

DNA Analysis Unit I
FBI Laboratory
935 Pennsylvania Ave. NW
Room 3845
Washington, D.C. 20535
(202) 324-6961

EDUCATION:

Master of Science (1995)
Physiology
Georgetown University
Washington, D.C.

Bachelor of Arts (1994)
Biology
University of Delaware
Newark, DE

PROFESSIONAL EXPERIENCE:

October, 1998 - Present	Forensic Examiner, DNA Analysis Unit I, FBI Laboratory Washington, D.C.
April, 1997 - October, 1998	Biologist, DNA Analysis Unit I, FBI Laboratory Washington, D.C.
October, 1995 - April, 1997	Biological Laboratory Technician, DNA Analysis Unit I, FBI Laboratory, Washington, D.C.

MEMBERSHIPS IN PROFESSIONAL AND SCHOLARLY ORGANIZATIONS:

1. American Academy of Forensic Sciences (2001)
2. Mid-Atlantic Association of Forensic Scientists (1996)
3. Phi Beta Kappa (1994)
4. Phi Kappa Phi (1993)
5. Golden Key National Honor Society (1992)

Curriculum Vitae

Jeffrey L. Leggitt, Supervisory Special Agent - Federal Bureau of Investigation

1984 B.S. Chemistry from West Texas State University

1989 MS, Pharmacology and Toxicology from University of Texas Medical Branch

1989 Research Associate, Baylor College of Medicine, Houston Texas

1991 Special Agent, Federal Bureau of Investigation,
Assigned to Albuquerque Division of the FBI, conducted various violent crime and Indian Country investigations.

1997 Supervisory Special Agent, Evidence Response Team (ERT) Unit,
FBI Academy, Quantico Va
coordinate ERT training and other administrative and operational matters

1999 Co-authored "The Presumptive Reagent Fluorescein for the Detection of Dilute Bloodstains and Subsequent STR Typing of Recoverable DNA", Journal of Forensic Sciences, 2000:45(5):1090-1092

The Presumptive Reagent Fluorescein for Detection of Dilute Bloodstains and Subsequent STR Typing of Recovered DNA

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Running Title: Fluorescein Detection and STR Typing

Contact author: Bruce Budowle

ABSTRACT

Luminol is the most commonly used presumptive reagent for identification of diluted or washed blood at a crime scene, and the use of luminol does not have a detrimental effect on subsequent DNA analysis. An alternate presumptive reagent for dilute blood detection is fluorescein. The sensitivity of fluorescein approaches the sensitivity of detection levels of luminol. The fluorescein detection method offers the advantages of working in a lighted environment, and the reaction persists longer than luminol. Degradative effects on DNA by fluorescein were tested. A series of diluted bloodstains, ranging from neat to 1:1,000,000, was placed on a variety of substrates. The substrates were: carpet, stained wood, unstained wood, black formica, white formica, cinder block, red brick, denim, and leather. Three sets were made per substrate. One set was exposed to fluorescein, one set was exposed to luminol, and one set served as an uncontaminated control. The fluorescein signal persisted longer than luminol. However, background staining for fluorescein was observed on some substrates within 30 seconds to one minute, and no background staining was observed for luminol. Stains on non-absorbent surfaces were detectable at 1:100,000 dilutions, and stains on absorbent surfaces were detectable usually at no more than 1:100. The sensitivity of detection of fluorescein was comparable to that of luminol in this study. In all cases, where sufficient DNA was recovered, typeable results at all 13 core CODIS STR loci were obtained from treated bloodstains and controls. The results from STR typing indicate that there was no evidence of DNA degradation. STR typing was possible on bloodstains up to the 1:100 to 1:1000 dilutions on non-absorbent surfaces (except for unstained wood which was typeable up to 1:50 to 1:100 dilutions), up to 1:50 to 1:100 dilutions on carpet and leather , on denim with neat samples and bloodstains diluted 1:10, and only neat blood samples on cinder

block. Red brick was the only substrate where the untreated sample was typeable at a greater dilution than the treated samples (1:100 dilution vs neat to 1:10 dilution, respectively). The results of the current study demonstrate that direct application of fluorescein (as well as luminol) on bloodstains does not interfere with the ability to recover typeable DNA and that the sensitivity of detection of the two presumptive reagents is comparable.

Key Words

Fluorescein, luminol, bloodstain, DNA, STR

INTRODUCTION

Dilute blood that can not be seen by eye, or blood on surfaces that have insufficient contrast with the blood, may be detected at crime scenes using a presumptive reagent. Luminol is the most commonly used presumptive reagent for identification of diluted (or washed) blood at a crime scene (1-7). Furthermore, the use of luminol does not have a detrimental effect on subsequent DNA analysis (2,4,5,7). Although very sensitive, luminol has certain operational limitations, which are: 1) the luminol reaction must be observed in as dark as possible an environment, making manipulations difficult, and identification relies on one's eyes becoming accustomed to the dark; and 2) the reaction is short-lived, usually lasting only a few seconds. Thus, photography, which may require long film exposures, necessitates additional treatments with luminol to maintain a positive reaction. Additional treatments may obliterate bloodstain patterns and may dilute the sample.

Cheeseman and DiMeo (8) recommend the presumptive reagent fluorescein for dilute blood and non-contrasted detection. The sensitivity of fluorescein approaches the sensitivity of detection levels of luminol. The fluorescein detection method offers the advantages of working in a lighted environment, and the reaction persists longer than that of luminol. However, no data exist on whether or not fluorescein has any detrimental effects on DNA, particularly analysis of Short Tandem Repeat (STR) loci. Before employing a reagent for blood detection, potential destructive effects on DNA should be assessed. Therefore, a study was carried out to compare fluorescein and luminol detection on a series of diluted bloodstains placed on a variety of substrates and to evaluate the ability to type STRs derived from samples treated with the presumptive test reagents.

MATERIALS AND METHODS

Blood from a single donor, drawn by venipuncture into an EDTA tube, was diluted 1:10, 1:50, 1:100, and 1:1000 with physiological buffered saline, and in some cases blood was diluted 1:10,000, 1:100,000, and 1:1,000,000. One hundred μl aliquots of neat and diluted blood (one set) were deposited onto various substrates and allowed to dry. The substrates were: carpet, stained wood, unstained wood, black formica, white formica, cinder block, red brick, denim, and leather. Three sets were made per substrate. The samples were maintained at ambient temperature for seven days. One set was used for exposure to fluorescein, one set was used for luminol, and one set served as an untreated control.

Luminol was purchased from Morris-Kopec Forensics, Inc. (Altamonte Springs, FL) and prepared according to manufacturer's recommendation. Fluorescin, the reduced state, was prepared fresh according to Cheeseman and DiMeo (8). Using an aerosol sprayer (Croion Industrial Products, Hebron, IL), each test reagent was applied to the stains such that the entire surface of the stain was covered. Fluorescein stained materials were observed using an alternate light source (450 nm) and orange safety goggles. Luminol stained materials were viewed in the dark with the naked eye.

Blood samples were recovered either by cutting or by swabbing with swabs wetted with distilled water. DNA was extracted according to Comey, et al (9). The quantity of recovered DNA was determined using the slot blot hybridization assay described by Waye, et al. (10) and Budowle, et al. (11). Amplification of the CODIS core 13 STR loci was carried out using the AmpFℓSTR Profiler Plus™ PCR Amplification Kit and the AmpFℓSTR COfiler™ PCR Amplification Kit (Perkin Elmer Biosystems, Foster City, CA) according to manufacturer's

recommendation. Typing was performed by capillary electrophoresis on the ABI Prism™ 310 Genetic Analyzer (Perkin Elmer Biosystems, Foster City, CA) according to the manufacturer's recommendations and Budowle and Moretti (12).

RESULTS AND DISCUSSION

In order to investigate the potential effects of the presumptive reagent fluorescein (as well as luminol) on STR analysis, bloodstains on absorbent (carpet, leather, denim), non-absorbent (stained wood, unstained wood, black formica, and white formica), and porous (brick and cinder block) materials were exposed directly to fluorescein or luminol. The positive attributes of using luminol are: 1) the preparation is commercially-available and requires a single application for initial viewing; 2) the preparation of the luminol reagent requires only the addition of water and thus can be prepared in the field; 3) no background staining was observed in our study, and 4) stains on non-absorbent surfaces were detectable at the 1:100,000 dilution, while stains on absorbent surfaces were detectable usually at no more than a 1:100 dilution. Sensitivity levels were similar to those reported by Fregeau, et al. (7). Less desirable attributes of the luminol method are: 1) manipulation of spraying in specified areas is difficult in the dark; and 2) depending on the substrate, the luminol reaction lasted a few to 30 seconds.

In contrast to luminol: 1) the fluorescein field-ready reagent is not commercially available and requires some laboratory preparation not routinely performed by crime scene investigators. Because of the need for fresh reagent, fluorescein may not be applicable by all investigators as a field deployable tool; 2) visualization of fluorescein-treated bloodstains requires use of an alternate light source set at approximately 450 nm and orange safety goggles; 3) manipulation of

spraying was easier because of working under lighted conditions; and 4) the fluorescein reaction persisted longer than luminol. However, background staining was observed on some substrates (i.e., carpet, wood, cinder block, and denim) within 30 seconds to two minutes. Since a crime scene investigator will never know *a priori* which materials may fluoresce in the presence of fluorescein, identification of potential bloodstains should still be performed in an expeditious manner. Additionally, it is prudent practice to initially test an unstained portion of a substrate, when possible, for potential background staining prior to applying the presumptive reagent for bloodstain detection. Stains on non-absorbent surfaces also were detectable at 1:100,000 dilutions, and stains on absorbent surfaces were detectable usually at no more than 1:100 dilution. Thus, the sensitivity of detection of fluorescein was comparable to that of luminol. Finally, the use of a thickener in the fluorescein preparation was deemed necessary to reduce running and possible mixing of different stains in close proximity.

The main purpose of the study was to determine if DNA could be recovered from fluorescein-treated samples and if the DNA was typeable for STRs. The entire swab or entire stain cutting was extracted in each case. The quantitation of DNA by slot blot hybridization was performed to demonstrate the presence of recoverable DNA and to determine the quantity of sample for the PCR. The quantity of recoverable DNA was not determined exactly, because some variation in recovery of DNA was observed due to swabbing (some sample remained on the substrate in a number of cases) and efficiency of extraction from cuttings can vary; also there was not a sufficient number of samples at each dilution/substrate/treatment for the amount of extractable DNA to be reliably determined. Moreover, the main purpose of the study was to determine whether or not DNA degrades in the presence of fluorescein. Thus, trends on DNA

quantity were evaluated. Generally, the amount of DNA recovered was similar (compared at appropriate dilution) for fluorescein-treated, luminol-treated, and untreated samples. DNA typically was detected by slot blot hybridization up to a 1:100 dilution, except for that recovered from denim (approximately 1:10 dilution), cinder block (no detectable DNA at neat or any dilution), red brick (only neat), and unstained wood (approximately 1:10 dilution). Since no DNA was recovered from the untreated cinder block controls, most likely the negative results are due to blood seeping into pores in the substrate and not being readily recoverable by swabbing.

In all cases, where sufficient DNA was recovered, typeable results at all 13 STR loci were obtained, with no evidence of DNA degradation. Regardless of treatment or no treatment, STR typing was possible on non-absorbent surfaces up to the 1:100 to 1:1000 dilutions (except for unstained wood which was typeable up to 1:50 to 1:100 dilutions), on carpet and leather up to 1:50 to 1:100 dilutions, on denim with neat samples and 1:10 dilutions, and on cinder block with neat samples only. Red brick was the only substrate where the untreated sample was typeable at a greater dilution than the treated samples (1:100 dilution vs neat to 1:10 dilution, respectively). It can be expected that evidentiary material purposely or inadvertently contaminated with fluorescein or luminol can be successfully typed, as long as sufficient quantity and quality DNA is recovered.

Greater dilutions of blood than were typeable in this study may be analyzed successfully in practice. The quantity of DNA in each extracted sample was used to determine the volume of sample for the PCR. However, a maximum of 20 μl could be placed in a PCR following the protocol used in this study. No effort was made to concentrate the extracted sample to increase the amount of template DNA for the PCR. Thus, more dilute samples may be typeable by concentrating the sample.

In conclusion, presumptive tests/enhancement reagents are needed to detect some bloodstains. In deciding which enhancement reagent to employ, it is desirable to appreciate the utility and limitations of the assay. The results of the current study demonstrate that direct application of fluorescein (or for that matter luminol) on bloodstains does not interfere with the ability to recover typeable DNA. Either reagent, fluorescein or luminol, may be used as a enhancement reagent for bloodstain detection at a crime scene, although a commercially-available, stable fluorescein assay would be desirable. The choice of reagent should be based on operational preferences of the investigator. Also, because of successful STR typing results from DNA obtained from diluted and contaminated bloodstains, the data in our study provide additional support for the reliability of STR typing.

This is publication number 99-10 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the Federal Bureau of Investigation.

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